The plasma concentrations of estradiol-17β, 17α-hydroxy-20β-dihydroprogesterone, progesterone, 11-ketotestosterone, testosterone, and gonadotropin were measured in adult female and male coho salmon (Oncorhynchus kisutch) concomitantly with the determination of gonad maturation during the spawning season. In females with eggs showing premigrating (central) germinal vesicles, migrating germinal vesicles, or peripheral germinal vesicles, estradiol-17β concentrations were higher than those measured in females in which the eggs had undergone germinal vesicle breakdown or ovulation. Conversely, plasma levels of 17α-hydroxy-20β-dihydroprogesterone were low at the three earliest stages of oocyte development and then increased dramatically at germinal vesicle breakdown and ovulation. Testosterone, 11-ketotestosterone, and gonadotropin levels generally increased with maturity of the eggs. In males, the levels of the hormones were stable.
relative to those measured in females with the exception of an increase in 17α-hydroxy-20β-dihydroprogesterone concentrations in the plasma during milt production. Endocrine profiles were also sketched according to date. In females, plasma estradiol-17β was high in fish returning from the ocean to a seawater holding facility before dropping off precipitously in females sampled at the end of the run. Plasma 17α-hydroxy-20β-dihydroprogesterone followed the opposite pattern, being low in fish sampled early and high in fish sampled at the end of the run. No easily discernable patterns emerged from the profiles of the other steroids and gonadotropin, although some significant variation in the concentrations occurred. In males, the plasma concentrations of 11-ketotestosterone, testosterone, and 17α-hydroxy-20β-dihydroprogesterone increased during the course of the run with the latter hormone being high during milt production. Utilizing the data for hormone concentrations and date, a model can be constructed for predicting the maturation stage (i.e. status of the ovary) of the female.

When adult hatchery coho salmon return from the sea to their natal hatcheries, they often remain in raceways for extended periods of time before ripening. In an attempt to circumvent the significant mortality often associated with this condition, as well as to obtain eggs when needed for
various rearing strategies, adult female coho salmon were injected intraperitoneally with the luteinizing hormone-releasing hormone analogue (LH-RHa) des-Gly\text{10}[D-Ala\text{6}]LH-RH-ethylamide at various dosages to induce precocious final maturation. The mean number of days to ovulation in all groups receiving LH-RHa was significantly lower than that of saline-injected controls. Within 13 days of the initial injection, at least 84% of the fish receiving two injections spaced 3 days apart of LH-RHa at various dosages had ovulated, at least 67% of the fish receiving single injections of LH-RHa at two dosages had ovulated, whereas less than 50% of the controls had ovulated. In all groups receiving LH-RHa, the percent fertilization of the eggs was slightly less than that of the control group.
Endocrine Mediation of Reproduction in Coho Salmon (Oncorhynchus kisutch)

by

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I. GENERAL INTRODUCTION

Coho salmon, *Oncorhynchus kisutch*, are economically important. The success of a hatchery depends upon the establishment and maintenance of a broodstock. In order to meet the demand for salmon, hatcheries must develop flexible production strategies. Attempts have been made to understand and control various aspects of salmon physiology. The endocrinology of reproduction is one area of research offering potential tools for the use in hatchery practices.

A variety of hormones are considered to exert control over the intricate physiology of reproduction in fish (Donaldson 1973; Schreck 1974). In maturing fish, high concentrations of gonadotropin(s) have been found in the blood (Crim et al. 1973, 1975; Billard et al. 1977; Fostier et al. 1978; Hontela and Peter 1978). *In vitro* studies have demonstrated the importance of gonadotropin(s) in stimulating steroidogenesis (Kagawa et al. 1982; Nagahama and Kagawa 1982) and also promoting final maturation of the ovary (Jalabert 1976; Jalabert et al. 1978a; Sower and Schreck 1982a). The hypothalamus is presumed to have some control over the secretion of gonadotropin(s) as has been demonstrated in mammals (Schally et al. 1973).
The gonads of teleosts produce steroids (Arai and Tamaoki 1967; Idler 1969; Hoar and Nagahama 1978; van Bohemen and Lambert 1978; Nagahama et al. 1982) and many of these steroids are found in the plasma (Campbell et al. 1980). By convention, the determination of the plasma profiles of various steroids and gonadotropin(s) constitutes the best way to assess in vivo the dynamics and progress of sexual maturation in teleosts.

Initially, I described some of the endocrine changes during sexual maturation in coho salmon and correlated these changes with histological development of the gonads. I measured estradiol-17β, progesterone, 17α-hydroxy-20β-dihydroprogesterone, testosterone, 11-ketotestosterone, and gonadotropin in coho salmon throughout the period of final maturation.

I also developed a model that would allow the prediction of the maturational state of female coho salmon. A successful model would permit the determination of ripeness based on physiological characteristics rather than on secondary sexual characteristics.

Finally, I investigated the possibility of inducing precocious final sexual maturation in females by treatment with an analogue of a mammalian hypothalamic hormone.
II. Profiles of sex steroids and gonadotropin in the plasma of coho salmon, *Oncorhynchus kisutch*, during final maturation

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3Cooperators are Oregon State University, Oregon Department of Fish and Wildlife, and U.S. Fish and Wildlife Service.
II. Profiles of sex steroids and gonadotropin in the plasma of coho salmon, *Oncorhynchus kisutch*, during final maturation.

INTRODUCTION

A variety of hormones are considered to exert control over the intricate physiology of reproduction in fish (Donaldson 1973; Schreck 1974). Many investigators have found high concentrations of gonadotropin(s) from the pituitary in the blood of maturing fish (Crim *et al.* 1973, 1975; Billard *et al.* 1977; Fostier *et al.* 1978; Hontela and Peter 1978). *In vitro* studies have demonstrated the importance of gonadotropin(s) in stimulating steroidogenesis (Kagawa *et al.* 1982; Nagahama and Kagawa 1982) and also promoting final maturation in females (Jalabert 1976; Jalabert *et al.* 1978a; Sower and Schreck 1982a). The hypothalamus is presumed to have some control over the secretion of gonadotropin as has been demonstrated in mammals (Schally *et al.* 1973). King and Millar (1980) found a hypothalamic substance from fish which was immunoreactive with antisera raised against mammalian gonadotropin-releasing hormone and Sherwood *et al.* (1983) isolated and sequenced salmon gonadotropin-releasing hormone. Van der Kraak *et al.* (1984) recently showed that mammalian gonadotropin-releasing hormone and superactive analogues
were potent stimulators of gonadotropin secretion in coho salmon.

The gonads of teleosts produce steroids (Arai and Tamaoki 1967; Idler 1969; Hoar and Nagahama 1978; van Bohemen and Lambert 1978; Nagahama et al. 1982) and many of these steroids are found in the plasma (Campbell et al. 1980). There is some indication that the interrenal may also produce steroids which are important in sexual maturation in some teleosts (Sundararaj and Goswami 1966a,b). By convention, the determination of the plasma profiles of various steroids and gonadotropin(s) constitutes the best way to assess in vivo the dynamics and progress of sexual maturation in teleosts.

The first objective of this study was to describe the changes in circulating levels of specific hormones during sexual maturation in coho salmon and to correlate these changes to histological development in the gonads. Sower and Schreck (1982b) reported on the circulating levels of estradiol-17β, progesterone, and total androgens in coho salmon during the spawning season—the current study set out to elaborate upon this earlier work by measuring not only the concentrations of estradiol-17β and progesterone, but also the levels of 17α-dihydroxy-20β-dihydroprogesterone (17α,20β-dihydroxy-4-pregnen-3-one) and two androgens: testosterone and 11-ketotestosterone. In
addition, the plasma levels of gonadotropin were measured. 
Sower et al. (1982a) attempted to induce ovulation in coho salmon held in seawater using exogenous hormone treatment with little success and Sower and Schreck (1982b) found that the circulating levels of the various steroids differed in coho that were held in seawater from those that were held in freshwater; thus, it may be that the susceptibility of coho salmon to hormonally-induced ovulation may be in part compromised in seawater by an alteration in the internal endocrine milieu of each fish. The levels of the hormones described in the current study were measured in coho salmon held in both seawater and freshwater in order to determine if any differences occurred in the endocrine profiles of fish held in either environment.

A model was developed to predict the maturational state of female coho salmon. This model is based on the information gathered about the circulating concentrations of the hormones and also the concomitant determination of oocyte development during the final phases of sexual maturation. In addition, testicular development was measured in the males in order to compare the hormone levels between males at various stages of final maturation.
MATERIALS AND METHODS

The studies were conducted at the seawater facility of Oregon-Aqua Foods, Inc. (OreAqua), Newport, Oregon during 1981 and 1982. One group of fish was sampled at the freshwater facility of OreAqua in Springfield, Oregon in 1981. In addition, coho salmon returning to the State of Oregon's freshwater hatchery at Fall Creek in 1982 were also used in the study.

Seawater Studies. From late September (24th and 30th respectively) until late November (20th and 30th respectively) in 1981 and 1982, groups of about 20 (10 females and 10 males) coho salmon on each sampling day were killed and bled. On seven of the 10 sampling days in 1981 and eight of the nine sampling days in 1982, gonads were removed from the fish and fixed in buffered formalin (females 1981; females and males 1982) or Bouin's solution (1981 males). Blood from the caudal vessel was collected in lithium heparinized vacutainers and centrifuged; plasma was drawn off and stored at -20°C until analyzed for progesterone (P4), estradiol-17β (E2), 17α-hydroxy-20β-dihydroprogesterone (DHP), 11-ketotestosterone (11-KT), testosterone (T), and gonadotropin (GtH). The gonads were removed from the formalin or Bouin's fixative, washed with water, held in 50% ethanol (EtOH) overnight, and then
stored in 70% EtOH. Stages of oocyte maturation were determined by histological examination of the ovaries. The stages of oocyte maturation in adult coho salmon, as described by Sower and Schreck (1982b), were defined as follows: (I) premigrating or central germinal vesicle (CGV), (II) migrating germinal vesicle (MGV), (III) peripheral germinal vesicle (PGV), i.e. germinal vesicle against the chorion, (IV) germinal vesicle breakdown and coalescence of lipid drops (GVBD), and (V) ovulation. The testes were also examined histologically and rated according to the following criteria: (1) predominance of 1° and 2° spermatocytes; (2) predominance of spermatocytes and spermatids; (3) spermatocytes, spermatids, and spermatozoa present equally; (4) predominance of spermatocytes and spermatozoa; (5) predominance of spermatids and spermatozoa; (6) predominance of spermatozoa; (7) production of milt, i.e. males gave milt when squeezed gently on the abdomen.

**Freshwater Studies.** On 24 November 1981, a group of 10 adult females and 10 adult males were sampled at spawning at the Springfield facility of OreAqua. These fish had been transported by truck from the OreAqua seawater facility. The methods of plasma collection were the same as those described for the seawater studies.

In 1982, a study was initiated to monitor the dynamics
of the endocrine system in individual females held throughout final maturation. A group of 10 female coho salmon which had just entered the hatchery trap at Fall Creek were anesthetized with CO$_2$ gas, tagged in each operculum, and then an aliquot of blood was collected from the caudal vein of each fish in vacutainers which contained heparin. The fish were then returned to the hatchery raceway for approximately three days when they were checked for ripeness. An aliquot of blood was collected and if a fish had not ovulated, it was returned to the raceway and the process was repeated until ovulation. The blood samples were centrifuged; the plasma was drawn off and stored at $-20^\circ$ C until analysis.

**Assays.** The plasma levels of P4 and E2 from the fish sampled in 1981 were measured by radioimmunoassay (RIA) as described by Sower and Schreck (1982b) except that in the E2 assays, no $\gamma$-globulin was added to assay tubes. For the 1982 samples, the E2 assay was modified slightly to increase the sensitivity at higher concentrations. The plasma levels of DHP, 11-KT, and T were determined by RIAs set up for the first time in our laboratory using the same generalized procedure as the RIAs for the E2 and P4 (Sower and Schreck 1982b). Each blood sample was extracted twice with diethyl ether for E2, DHP, 11-KT, and T or hexane:benzene (2:1) for P4. Extraction recovery for each steroid was determined by spiking 10 tubes containing plasma at the respective assay
volume with 5000 or 10000 dpms (disintegrations per minute) of the appropriate $[^3\text{H}]$steroid in phosphate buffered saline-gelatin (PG) and putting each tube through the extraction procedure. The procedure entailed adding 1.5 ml of extraction solvent to the plasma, vortexing, freezing the aqueous phase in liquid $N_2$, decanting and combining the two extracts, and drying under $N_2$ gas or air. For P4, E2, and DHP the replicate extracts were reconstituted each with 0.2 ml of PG (pH=7.0). For extraction recovery efficiency, 0.05 ml PG-extract solution was removed to a scintillation vial and counted. For 11-KT and T assays, 1 ml of PG was added to each extract tube and two 0.1 ml aliquots were removed for the 11-KT assay (with 0.1 ml of PG added) and two 0.2 ml aliquots were removed for the T assay. For all assays, each tube of extract and each standard tube received 0.1 ml of the appropriate antiserum in PG (at the proper dilution)—the standard tubes contained steroid in a range of concentrations from 3.8 to 2000 pg. To each tube was added 0.1 ml of the appropriate $[^3\text{H}]$steroid (10000 dpms in PG). The tubes were incubated at room temperature for 90 minutes, placed in an ice bath for 5 minutes, and then 1.0 ml of dextran charcoal suspension (2.5 g charcoal and 250 mg Dextran in 1.0 liter of phosphate buffer saline) was added to each tube in the P4, DHP, 11-KT, and T assays or 0.5 ml of dextran charcoal suspension (6.25 g charcoal and 4.00 g
Dextran in 1.0 liter PG) was added to each tube in the E2 assay. The tubes were incubated for 15 minutes in an ice bath, centrifuged at 2200 g for 20 min, and then a 0.5 aliquot was decanted into Budget-Solve® (Research Products International Corp., Mount Prospect, IL) scintillation fluid and counted in either a Packard liquid scintillation spectrophotometer or a Beckman LS 1800 liquid scintillation system.

Progesterone was determined in 200 µl of plasma or serum. Antiprogestosterone antisera (11-BSA) was obtained from Dr. G. Niswender (Colorado State University, Fort Collins, Colorado) and diluted to 1:2500 in PG. The interassay coefficient of variation for the P4 RIA of coho plasma was 16.3%. The lower limit of detection was about 7.8 pg per tube.

Estradiol-17β was determined in 100 µl of plasma from all fish in 1981 and males in 1982, and 25 µl of plasma from females in 1982. Antiestradiol-17β antisera (S-244) was obtained from Dr. G. Niswender and diluted to either 1:60,000 (1981) or 1:7500 (1982) in PG. The antisera used on the 1981 samples was from a batch obtained in 1979. The intra-assay and interassay coefficients of variation for the E2 RIA were 8.1% (n=5) and 19.8% (n=5) respectively. The lower limit of detection was about 3.9 pg per tube.

The following assays were set up for the first time in our laboratory--each assay was verified using thin-layer
chromatography. 17α-hydroxy-20β-dihydroprogesterone was determined in 25 μl (or 10 μl for expected high values) of plasma. Anti-17α-hydroxy-20β-dihydroxprogesterone antisera was obtained as a gift from Dr. A.P. Scott (Ministry of Agriculture, Fisheries Laboratory, Lowestoft, Suffolk, United Kingdom) and diluted 1:100,000 in PG. The intra-assay and interassay coefficients of variation for the DHP assay were 5.9% (n=6) and 12.9% (n=8) respectively. The cross-reactivity with cortisol, 17α-hydroxyprogesterone and P4 was less than 0.1% (Scott et al. 1982).

11-ketotestosterone was determined in an equivalent of 2.5 μl of plasma (diluted from 25 μl plasma). Anti-11-ketotestosterone antisera was obtained as a gift from Dr. Y. Nagahama (Laboratory of Reproductive Biology, National Institute for Basic Biology, Okazaki, Japan) and diluted 1:50,000. The intra-assay and interassay coefficients of variation for the 11-KT assay were 6.8% (n=7) and 10.9% (n=7) respectively. The crossreactivity with 11β-hydroxyltestosterone was 5.5% (Y. Nagahama, pers. comm.) and with T was 1.8%.

Testosterone was determined in an equivalent of 5.0 μl (diluted from 25 μl of plasma) of plasma. Antitestosterone antisera (T3-125) was obtained from Endocrine Sciences (Tarzana, California) and diluted 1:1,020. The intra-assay and interassay coefficients of variation for the T assay
were 7.8% (n=8) and 13.7% (n=5) respectively. The crossreactivity with 11-KT was about 4.7%.

Gonadotropin was determined by RIA with the assistance of Glen van der Kraak, Helen Dye, and Dr. Edward Donaldson (West Vancouver Laboratory, Fisheries and Oceans, West Vancouver, British Columbia). The assay used an antiserum directed against chinook salmon GtH (see van der Kraak et al. 1983).

Statistics. Data for hormone concentrations were analyzed by a Student-Newman-Keuls test, which computes the significances of differences for samples of unequal sizes after preliminary analysis of variance. In all tests, the level of significance was P<0.05. The model for determining maturity from date and hormone concentrations was constructed with a multiple regression statistical computer package (Nie et al.). A stepwise regression procedure in which variables were added in the order of significance was employed to construct the final models. A lack of fit test was used to determine the significance (p<0.05) of the addition of each variable to the model (Snedecor and Cochran 1980).
Figure 1. Plasma concentrations (ng/ml) of estradiol-17β (E2) and 17α-hydroxy-20β-dihydroprogesterone (DHP) during final sexual maturation in female coho salmon sampled in seawater (1981 and 1982) and in freshwater (1981). Each value represents the mean and standard error of the indicated number of samples. Plasma levels are presented in relation to both date sampled (a,c) and maturation stage (I=premigrating germinal vesicle; II=migrating germinal vesicle; III= peripheral germinal vesicle; IV=germinal vesicle breakdown; and V=ovulation) of the ovary (b,d).
Figure 1.
RESULTS

Females. The circulating levels of E2 tended to decrease during the spawning season. The plasma concentrations of E2 in most of the female fish that entered the seawater recapture facility between 24 September and 12 November in 1981 were elevated in comparison to the concentrations found in the fish that entered later on 17 and 20 November and in the fish which were spawned on 24 November in freshwater (at Springfield). In 1982, the plasma concentrations of E2 in females entering the facility remained elevated throughout the sampling period until 30 November when the levels dropped precipitously (Fig. 1a). Although a downward trend in the concentrations of the steroid is apparent in Figure 1a, the variation between individuals is pronounced. In 1981, only the mean concentrations from females sampled on 24 and 30 September and 19 and 29 October were significantly different (statistically) from the mean concentrations of fish sampled on 17, 20, and 24 November. If the data for 1981 are examined with regard to maturation state of the ovary instead of the date upon which the fish were sampled, females in which the eggs had migrating germinal vesicles (MGVs) or peripheral germinal vesicles (PGVs) had significantly higher plasma concentrations of E2 than those
of fish in which the eggs showed germinal vesicle breakdown (GVBD) or in which ovulation had occurred. Similarly in 1982, females in which the eggs had central germinal vesicles (CGVs), MGVs, or PGVs had plasma concentrations of E2 higher than those of fish in which the eggs had undergone GVBD or ovulation (Fig. 1b).

In contrast to E2, the circulating levels of DHP increased during the spawning season. The plasma concentrations of DHP were low in females sampled in September and October (1981) and were very high in females sampled on 17 and 20 November in seawater and on 24 November in freshwater at Springfield (Fig. 1c). The plasma concentrations of DHP started to increase in females sampled in mid-November in 1981, although the variability in levels on some sampling dates was considerable. The 10 fish sampled on 2 November had a wide range of plasma DHP values (2.8 to 76.6 ng/ml), whereas the three females sampled on 12 November all had low plasma levels of the steroid. All of the females sampled in freshwater at Springfield (24 November 1981) had high plasma concentrations of DHP. From the end of September until 20 October in 1982, the plasma concentrations of DHP remained low in females entering the facility. Individual females began showing elevated plasma titers of the hormone between 25 October and 10 November. By the end of November, the mean plasma level of DHP were
Figure 2. Plasma concentrations (ng/ml) of 11-keto-testosterone (11-KT) and testosterone (T) during final sexual maturation in female coho salmon sampled in seawater (1981 and 1982) and in freshwater (1981). Each value represents the mean and standard error of the indicated number of samples. Plasma levels are presented in relation to both date sampled (a,c) and maturation stage (I=premigrating germinal vesicle; II=migrating germinal vesicle; III=peripheral germinal vesicle; IV=germinal vesicle breakdown; and V=ovulation) of the ovary (b,d).
Figure 2.
significantly higher than any earlier group (Fig. 1c). The mean concentrations from females in which GVBD or ovulation had occurred were significantly higher than those from females in which the eggs had CGVs, MGVs, or PGVs in both 1981 and 1982 (Fig. 1d). In 1982, females with PGVs had significantly higher levels of DHP than did females with oocytes containing CGVs or MGVs.

The levels of 11-KT tended to be variable throughout the run. The plasma concentrations of 11-KT in females ranged from 0.6 to 21.7 ng/ml. No readily discernable pattern emerged from viewing the hormone concentrations relative to date in 1981 except that the females which were spawned in freshwater on 24 November had levels of 11-KT significantly higher than those of females sampled in September, on two dates in October, and on two dates in November (Fig. 2a). In 1982, the mean plasma levels of 11-KT did not differ significantly between females sampled on the various dates, although the data hint of a trend towards higher levels as the season progressed. 11-KT appears to increase with degree of maturation. In 1981, this trend is apparent as ovarian development progressed, although not statistically significant. In 1982, the mean concentration of 11-KT from females containing ovulated eggs was significantly higher than the mean levels found in females with CGVs or MGVs (Fig. 2b).

In general, the plasma levels of T tended to increase
in females (although not statistically significant) over the period of the run and were an order of magnitude higher than the concentrations of 11-KT. The concentrations were low in females sampled in late September (1981) and showed a trend of higher levels thereafter (except for the three females sampled on 20 November, all of which had low levels of T). The variation between individuals was pronounced and only the levels of T from females that were sampled in freshwater on 24 November were judged significantly higher than the levels found in fish sampled in September (Fig. 2c). A slight upward trend can be seen in the mean plasma levels of T from females sampled on the various dates in 1982, although no significant differences were found. In 1981, each successive stage of maturation showed higher mean plasma levels of T and in fish which had ovulated, the mean plasma concentration of T was significantly higher than that of females in which the eggs had MGVs. In 1982, females in which the eggs contained CGVs had significantly lower levels of T than females with PGVs (Fig. 2d). In spite of individual variation which precluded the identification of significant differences between other maturity levels, it is apparent that the females in which the eggs had PGVs, GVBD, or in which ovulation had occurred had higher mean plasma concentrations of T than the means from females with CGVs or MGVs.

The mean plasma levels of P4 did not differ
Figure 3. Plasma concentrations (ng/ml) of progesterone (P4) and gonadotropin (GtH) during final sexual maturation in female coho salmon sampled in seawater (1981 and 1982) and in freshwater (1981). Each value represents the mean and standard error of the indicated number of samples. Plasma levels are presented in relation to both date sampled (a, c) and maturation stage (I = premigrating germinal vesicle; II = migrating germinal vesicle; III = peripheral germinal vesicle; IV = germinal vesicle breakdown; and V = ovulation) of the ovary (b, d).
Figure 3.
significantly between sampling dates or between maturation stages in females (Figs. 3a,b). The plasma concentrations ranged from 0.1 to 1.1 ng/ml and remained constant throughout the season and also through oocyte development.

The plasma concentrations of GtH were extremely variable in females sampled in seawater in 1982. Although the mean levels of GtH on 10 and 30 November were much higher than those from the other dates (Fig. 3c), the mean concentrations did not differ significantly between sampling dates. The mean plasma concentrations of GtH were higher for females in which the oocytes had PGVs, GVBD, or ovulated than those of females with CGVs or MGVs; however, only the PGV group had GtH levels significantly higher than the MGV group (Fig. 3d).

No significant differences were found in the means of any hormones from females at the same maturation stage which had entered the seawater facility at various times throughout the run. In other words, a female with MGVs entering in September had similar plasma levels of the various steroids and GtH as a female with MGVs entering in October or November.

The plasma concentrations of E2, DHP, 11-KT, T, and GtH in females sampled in freshwater at Fall Creek hatchery were similar to those found in the more mature (i.e. GVBDs or ovulated) females sampled in seawater. The mean plasma concentrations of E2, DHP, 11-KT, T, and GtH in females
Figure 4. Plasma concentrations (ng/ml) of: a) estradiol-17β (E2), 11-ketotestosterone (11-KT), and gonadotropin (GtH); and b) 17α-hydroxy-20β-dihydroprogesterone (DHP) and testosterone (T); in female coho salmon sampled in freshwater at Fall Creek Hatchery in 1982. Each value represents the mean and standard error of the indicated number of samples.
Figure 4.

PLASMA CONCENTRATION (ng/ml)

DAYS BEFORE OVULATION
Figure 5. Plasma concentrations (ng/ml) of estradiol-17β (E2) and 17α-hydroxy-20β-dihydroprogesterone (DHP) during final sexual maturation in male coho salmon sampled in seawater (1981 and 1982) and in freshwater (1981). Each value represents the mean and standard error of the indicated number of samples. Plasma levels are presented in relation to both date sampled (a,c) and maturation stage (I=predominance of 1° and 2° spermatocytes; II=predominance of spermatocytes and spermatids; III=spermatocytes, spermatids, and spermatozoa present equally; IV=predominance of spermatocytes and spermatozoa; V=predominance of spermatids and spermatozoa; VI=predominance of spermatozoa; and VII=milt production, i.e. males gave milt when squeezed gently on the abdomen) of the testes (b,d).
Figure 5.
sampled in freshwater at the Fall Creek hatchery are shown in Figure 4. By noting the time of ovulation, the days prior to ovulation were back-calculated and thus, the hormone dynamics in the circulation over the two weeks before ovulation were followed. Although none of the mean plasma levels of the hormones showed any significant changes, some trends did emerge in the individual fish. In nine of 10 females, plasma E2 levels were lower and GtH levels were higher at ovulation than prior to ovulation. Plasma concentrations of DHP, 11-KT, and T fluctuated before ovulation, but not in any readily discernable pattern.

Males. The plasma levels of E2 in males were low in comparison to those measured in females. The mean concentrations remained constant in males sampled in seawater and in males sampled in freshwater at Springfield in 1981. (Fig. 5a). Similarly, the mean levels of E2 in males sampled in 1981 did not differ significantly when viewed according to maturation state of the testes, although a slight drop occurred in males with testes predominated by spermatozoa and in males which were producing milt. In 1982, males which were taken at the end of the run and males which were producing milt had mean plasma E2 levels lower than the mean concentrations from any other date or from any other maturity group (Fig. 5b). The concentration of DHP increased in males as the spawning run progressed. The mean plasma concentrations of
Figure 6. Plasma concentrations (ng/ml) of 11-keto-testosterone (11-KT) and testosterone (T) during final sexual maturation in male coho salmon sampled in seawater (1981 and 1982) and in freshwater (1981). Each value represents the mean and standard error of the indicated number of samples. Plasma levels are presented in relation to both date sampled (a,c) and maturation stage (I=predominance of 1° and 2° spermatocytes; II=predominance of spermatocytes and spermatids; III=spermatocytes, spermatids, and spermatozoa present equally; IV=predominance of spermatocytes and spermatozoa; V=predominance of spermatids and spermatozoa; VI=predominance of spermatozoa; and VII=milt production, i.e. males gave milt when squeezed gently on the abdomen) of the testes (b,d).
Figure 6.
DHP in males entering the seawater facility on 17 and 20 November and in males spawned in freshwater on 24 November were significantly higher than those of males sampled on any other date during the 1981 run. In 1982, males sampled on 5 and 30 of November had mean levels significantly higher than those of males sampled in September or October (Fig. 5c). In both 1981 and 1982, males which were producing milt had a mean concentration higher (although not significantly for 1981) than that of any other group of males. Males with testes containing predominantly spermatozoa had mean levels of DHP higher than those of males with any less developed testes in both years (Fig. 5d).

In general, the mean plasma concentrations of 11-KT in males showed an upward trend as time progressed. In 1981, the mean plasma 11-KT level was significantly higher in males sampled on 17 November in seawater and 24 November in freshwater at Springfield than those of males sampled in late September. In 1982, males sampled in November and the latter part of October had higher mean levels than those found in males sampled between 30 September and 8 October (Fig. 6a). Plasma concentrations of 11-KT were higher in males than in females and tended to increase with time (in 1981 and 1982) and maturation state (in 1982) of the testes (Fig. 6b).

In both 1981 and 1982, males sampled at some point before the end of the run in the seawater facility had the
Figure 7. Plasma concentrations (ng/ml) of progesterone (P4) and gonadotropin (GtH) during final sexual maturation in male coho salmon sampled in seawater (1981 and 1982) and in freshwater (1981). Each value represents the mean and standard error of the indicated number of samples. Plasma levels are presented in relation to both date sampled (a,c) and maturation stage (I=predominance of 1⁰ and 2⁰ spermatocytes; II=predominance of spermatocytes and spermatids; III=spermatocytes, spermatids, and spermatozoa present equally; IV=predominance of spermatocytes and spermatozoa; V=predominance of spermatids and spermatozoa; VI=predominance of spermatozoa; and VII=milt production, i.e. males gave milt when squeezed gently on the abdomen) of the testes (b,d).
Figure 7.
highest mean concentrations of plasma T. In 1981, the males sampled on 22 October in seawater and sampled in freshwater on 24 November had mean levels significantly higher than those of any other group. In 1982, males sampled on 5 October had a mean concentration significantly higher than the means from six of the eight other groups sampled in seawater (Fig. 6c). The plasma levels of T in males were generally lower than those found in females and were always lower than the levels of 11-KT found in the same males. No significant differences were found in the means from the males of the various maturity groups in either 1981 or 1982 (Fig. 6d).

The mean plasma levels of P4 did not differ significantly between groups of males differentiated by either date or testes maturity (Figs. 7a and 7b) in 1981. Neither groups sampled on different dates nor groups with the various stages of maturity had mean plasma concentrations of GtH which were different from other respective groups of males (Figs. 7c and 7d). However, the plasma levels of GtH were generally higher in males throughout the run than those in all but the latest entering females.
DISCUSSION

**Females.** From the endocrine profiles, a model was constructed to predict maturity state of the females. The general form of the model is:

\[ Y = B_0 + B_1 \log(DHP) + B_2 D - B_3 \log(E2) \]

where \( Y \) is the maturity state (1=CGV, 2=MGV, 3=PGV, 4=GVBD, and 5=ovulation), DHP and E2 are the plasma concentrations of DHP and E2 respectively, \( D \) is the number of days from 1 January to the sampling date, and \( B_0, B_1, B_2, \) and \( B_3 \) are the multiple regression coefficients. For the 1981 data, the addition of \( B_4 \log(T) \) (where \( T \) is the plasma concentration of \( T \)) significantly (P<0.05) increased the fit of the model. Separate multiple regression models were constructed for each year. The coefficients of determination for 1981 and 1982 were 0.77 and 0.84 respectively—-in other words, 77% and 84% of the total variation observed in the maturity state of females could be accounted for by the respective models.

Specifically, the models constructed for each spawning run were:

(1981) \[ Y = -6.59 + 0.72 \log(DHP) + 0.02D - 0.72 \log(E2) + 0.83 \log(T) \]

(1982) \[ Y = -3.67 + 1.09 \log(DHP) + 0.02D - 0.62 \log(E2) \]
Examination of the regression coefficients reveals that logDHP, D, and logT are positively correlated with maturity state whereas logE2 is negatively correlated.

This model depicts the strong correlation between the endocrine environment, as reflected by the plasma concentrations of the various hormones, and the development of the ovary, as reflected by the distinct histological stages of the eggs. The model may have commercial importance as the ability to manipulate the physiology of reproduction in females (e.g. accelerate maturation, induce ovulation) depends on the sensitivity of the females to treatment, i.e. successful induction of ovulation using exogenous hormones may depend not only on the dosage and number of treatments, but also on the endocrine status and ovarian development of the females. Thus, determining the levels of specific steroids in the plasma from a population may allow for a prediction of the sensitivity of that population to treatment with maturation-inducing hormones.

Sower and Schreck (1982b) found that the gonadosomatic index (GSI), i.e. the percentage of the gonad weight in relation to the somatic weight, in coho salmon increased over the course of the spawning run. In rainbow trout, Salmo gairdneri, increasing GSI has been positively correlated to an increase in plasma vitellogenin (van Bohemen et al. 1981)—the yolk protein—which in turn has
been correlated to elevated E2 concentrations in the plasma (van Bohemen and Lambert 1981; Bromage et al. 1982). Rising levels of plasma E2 and vitellogenin have been reported to parallel increases in ovarian yolk lipophosphoprotein in female brown trout *S. trutta* (Crim and Idler 1978). The elevated plasma titers of E2 in female coho salmon early in the run and in fish with egg maturity stages of CGV, MGV, and PGV suggest that these fish are undergoing vitellogenesis. Several steroids have been reported to bind to specific regions of the brain (Kim et al. 1978; Demski and Hornby 1982). The high concentrations of E2 in the plasma generally correspond to low levels of plasma GtH, supporting the contention that in mature teleosts E2 inhibits the secretion of GtH through negative feedback on the hypothalamus and/or pituitary (Billard and Peter 1977). With the end of vitellogenesis comes the onset of GVBD and finally ovulation—events which do take place in coho salmon held in seawater, but evidence suggests that fertility may be impaired through dysfunction in the regulation of ions and osmolality (Sower and Schreck 1982b). The low plasma levels of E2 at these final stages of oocyte maturation indicate that steroid production has been shifted away from the aromatization pathway and the high GtH levels suggest that the negative feedback of E2 to the brain has been curtailed or that low levels of E2 exert a positive influence upon GtH secretion.
Since the isolation of DHP from the plasma of sockeye salmon, *O. nerka*, (Idler *et al.* 1960), this hormone has been found in high concentrations in the plasma of rainbow trout (Campbell *et al.* 1980; Scott *et al.* 1983), amago salmon, *O. rhodurus*, (Young *et al.* 1983, Ueda *et al.* 1983), and chum salmon, *O. keta*, (Ueda *et al.* 1984). *In vitro* studies have shown that this steroid is a potent inducer of final maturation in a number of teleosts (Jalabert 1976; Jalabert *et al.* 1978a,b; Suzuki *et al.* 1981; Sower and Schreck 1982a; Nagahama *et al.* 1983). The high levels of DHP in the plasma of coho salmon at GVBD and ovulation suggest that this steroid is important during these two phases of egg development, although *in vitro* research has shown that it cannot induce ovulation without the addition of GtH (Jalabert *et al.* 1978a).

In fact, plasma concentrations of GtH were elevated in females which had ovulated and in which the eggs had PGV or GVBD; however, the individual variation in concentrations was pronounced—suggesting that maximal secretion of GtH may not be required for final maturation. In addition, the extent of the variation in the capacity of individual females to produce and secrete GtH is unknown. Despite the variability between concentrations in individuals, the plasma levels of GtH in females sampled over the period of final maturation in fresh water did follow a pattern, i.e. GtH concentrations were higher at ovulation than before
ovulation in 9 of 10 fish.

Although the plasma concentrations of DHP increase dramatically during final maturation, this steroid may not be the final inducer of maturation. The oocyte maturation of amphibians is also stimulated by a progestin—P4—which acts by binding to the surface of the egg (Masui and Markert 1971) and stimulating the production of a maturation-promoting factor within the egg (Masui and Clarke 1979; Masui and Markert 1971; Maller and Krebs 1977; Kanatani and Nagahama 1980). The process in teleosts may be analogous. In addition, prostaglandins have been implicated as inducers of ovulation in teleosts (Jalabert 1976; Cetta and Goetz 1982; Stacey and Goetz 1982). The high plasma concentrations of DHP in coho salmon may indicate that this steroid has more influence than just on the maturation of oocytes, but it is unknown if and how DHP affects, for instance, the central nervous system.

The plasma dynamics of T and 11-KT seem to "anticipate" the increase in plasma DHP concentrations during the final stages of oocyte development. Despite the concentrations of T being about 10-fold higher than those of 11-KT, both androgens show the same profile of rising throughout oocyte maturation. Scott and Sumpter (1983) suggest that the high T levels in female rainbow trout are due to superactivity of the ovarian steroidogenic cells during oocyte maturation. They postulate that the rapid rise in 17α-hydroxylated
progestagens (i.e. DHP and 17α-hydroxyprogesterone) just before ovulation in trout occurs through the blockage of ovarian androgen synthesis. However, in coho salmon, apparently no blockage occurs as both T and 11-KT concentrations continue to rise in the plasma through ovulation. Testosterone production by the thecal layer of the ovarian follicle has been reported to be a necessary step in estrogen synthesis by the granulosa cells (Kagawa et al. 1982; Nagahama et al. 1982; Young et al. 1982); however, T levels in the plasma are 10-fold higher than those of E2 and T levels in the plasma are high throughout the maturation period whereas E2 concentrations fall off precipitously as ovulation approaches. Testosterone may perform other roles besides that of serving as a reservoir for the production of other steroids. Crim and Evans (1979) demonstrated that treatment of juvenile rainbow trout with T resulted in increased concentrations of pituitary GtH, Soivio et al. (1982) showed that T was metabolized in the skin of brown trout, Sower et al. (1983) showed that 17-methyltestosterone increased skin thickness, and Nagahama et al. (1980) reported that although not as effective as DHP, T could induce GVBD in amago salmon and rainbow trout. Testosterone was particularly elevated in the plasma of female coho salmon spawned in freshwater which may be indicative of a function during ovulation or a role in osmoregulation.
The role of 11-KT in females is not known. The importance of 11-KT during the final maturation of females has been discounted because of low or non-detectable levels in the plasma of rainbow trout (Scott et al. 1980a) and the winter flounder, Pseudopleuronectes americanus (Campbell et al. 1976); however, the levels of this steroid in the plasma of Pacific salmon reported previously (Idler et al. 1961) and here, suggest that its function may be largely uninvestigated rather than unimportant. Idler et al. (1961) found that injections of 11-KT increased skin thickness and coloration in female sockeye salmon. Testosterone and 11-KT may work synergistically with the other sex steroids to regulate the complex physiological and behavioral events which comprise final maturation and spawning in coho salmon.

The question of whether one or two GtHs exist makes interpretation of the GtH data difficult. Schulz et al. (1981) reported that GtH acts by increasing cyclic adenosinemonophosphate (cAMP) concentrations in the gonads of rainbow trout, and Fontaine-Bertrand et al. (1978) showed that incubating pieces of ovaries of eel, Anguilla anguilla, with carp GtH increased the levels of cAMP in the media. It may be through the production of cAMP that the elevated concentrations of GtH observed in maturing coho salmon females act to increase the ovarian synthesis and release of steroids, prostaglandins, and/or the maturation-promoting
factor.

The mean levels of the various hormones in females held in freshwater at Fall Creek throughout the 2 week period ending at ovulation corresponded to the mean levels found in females which entered the seawater facility at Newport late in the run, i.e. mid to late November. This suggests that by the time the Fall Creek females were sampled (late October), vitellogenesis was complete and GVBD had commenced. The magnitude of individual variation in hormone levels suggests that absolute plasma concentrations may not be exclusively important in the determination of maturation status, which may explain the prominent position date has in the model presented above.

Males. Since all the males that underwent histological examination of the testes had spermatozoa present and the majority of males sampled in both years were producint milt or had spermatozoa as the most prevalent cell type in the testes, it appears that males entering the seawater facility were near the end of spermiogenesis. This reproductive "strategy" means that the reproductive physiology of the male must essentially "wait" for that of the female to undergo final maturation. This situation is evident in salmonids in general where the males typically ripen before the females. It is not surprising that most of the hormone levels showed stability relative to the profound changes observed in those of the females.
Of all the steroids measured, DHP showed the most prominent changes during maturation in the males. The high concentrations late in the run and in males which were producing milt supports the contention that DHP plays an important role in the process of milt production in salmonids (Scott and Sumpter 1983; Ueda et al. 1983).

The levels of 11-KT in the plasma were higher than those of T in the males which is consistent with observations made in other salmonids (Campbell et al. 1976, 1980; Idler et al. 1971; Scott et al. 1980b; Hunt et al. 1982). Scott et al. (1980b) suggested that 11-KT played a major role in spermiation (i.e. milt production) in rainbow trout; however, no major increase in the plasma concentrations of this steroid occurred in coho salmon which were producing milt. The concentrations in males were elevated throughout the run--generally higher at the end than at the beginning--implying that 11-KT may play some role in the development of spermatocytes into spermatozoa and perhaps the maintenance of spermatozoa. The influence of 11-KT may not be limited to testicular development. Idler et al. (1961) reported that treatment of male sockeye salmon with 11-KT increased skin thickness, elongation of the snout, and redness of the skin.

The mid-run peak in the mean plasma concentrations of T did not correspond to any particular maturity level in males.
although the levels appeared higher prior to spermiation. Scott et al. (1980b) suggested the possibility that the presence of T in the plasma of male trout may be incidental because it is one of the intermediate products in the synthesis of 11-KT; however, T has been shown to have a variety of other effects including stimulation of GtH synthesis in juvenile rainbow trout (Crim and Evans 1979) and it is known to be metabolized by the skin of brown trout (Soivio et al. 1982). The high levels of T observed in males held in freshwater during the production of milt relative to those in males of the same maturity held in seawater may indicate an osmoregulatory role for this steroid in freshwater or endocrine dysfunction in fish retained in seawater. Billard et al. (1977) demonstrated that castration of maturing rainbow trout resulted in increased GtH levels in the plasma, thus suggesting the existence of negative feedback by either T or 11-KT, and Demski and Hornby (1982) reported that teleosts concentrate tritiated T in areas of the brain. Thus the role of T in physiology other than spermiogenesis may not be insignificant.

In the literature, the plasma levels of E2 in males is largely ignored due to the lack of prominence of this steroid relative to other steroids in males and relative to levels found in females. Despite the low concentrations and the change is assay protocol (which affected sensitivity of
the assay), males which were producing milt showed a
decrease in the plasma levels of E2 in comparison to males
at other stages of spermiogenesis for both years. Whether
this finding is coincidental or important is unknown.

Throughout the run, males had higher plasma levels of
GtH than did the females early in the run. The males
entering the seawater facility were already in a mature
state and therefore, baseline levels of GtH are probably
unobserved. The steady secretion over the course of the run
and at all the various maturity stages suggests that a
general stimulation by GtH of steroid production in the
testes is occurring. The lack of a peak in plasma GtH
corresponding to milt production once again raises
questions of the existence of a second GtH and of the need
for maximal secretion to influence milt production. Crim
et al. (1975) in a review article reported that the
pituitary is necessary for mitotic division of
spermatogonia, the transformation of spermatogonia into
primary spermatocytes, and spermiation in salmonids.

Most of the endocrine profiles of fish reported in the
literature present concentrations of hormones relative to
date of sampling. Such a practice carries the implication
that the populations under study were synchronous in
maturation—this is not the case with the coho salmon
sampled in this study, especially the females. The model
developed here had a high degree of success in predicting the maturational status of the oocytes through knowledge of specific endocrine parameters and date. It appears that the significance of certain changes in the plasma concentrations of hormones can be better understood by viewing these changes relative to the development of the gonads rather than simply by date.
III. LH-RHa induces precocious sexual maturation and ovulation in coho salmon

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III. LH-RHa induces precocious sexual maturation and ovulation in coho salmon.

INTRODUCTION

The acceleration of the final processes of sexual maturation in salmonids is desirable for various hatchery practices, and exogenous hormone treatments are effective for this purpose (Jalabert et al. 1978b, Hunter et al. 1981, Sower et al. 1982). The most successful treatment to date consists of an injection of partially purified salmon gonadotropin (SG-G100) or salmon pituitary extract (SPE) followed by an injection of the luteinizing hormone-releasing hormone analogue (LH-RHa) des-Gly^{10}[D-Ala^{6}]LH-RH-ethylamide. Although this treatment results in a significant acceleration of final maturation, the cost and difficulty of obtaining the salmon pituitary products make this treatment very expensive for use on a production scale.

In this study, we determined whether LH-RHa could be used alone to induce ovulation in coho salmon, thereby obviating the need for exogenously administered salmon pituitary products. We evaluated the effects of LH-RHa at various doses when administered in single or in two serial injections. Since the technique of administering SG-G100 or SPE followed by LH-RHa requires handling the fish several times, which causes stress, we also investigated the
possibility of using a single combined injection of SPE and LH-RHa to accelerate maturation.
TABLE I.

Ovulatory response of coho salmon after treatment with lyophilized pituitary extract (SPE) and luteinizing hormone-releasing hormone analogue (LH-RHa), different doses of LH-RHa, or saline. Injection protocol, mean number of days to ovulation, and mean percent fertilization of the eggs.

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<th>Percent fertilization</th>
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MATERIALS AND METHODS

Adult coho salmon from the seawater facility of Oregon Aqua Foods, Inc. at South Beach, Oregon, were transported by truck to the freshwater hatchery at Springfield, Oregon. On October 15, 1982, adult female coho salmon exhibiting coloration indicative of advanced sexual maturity were sorted from the hatchery broodstock population. The fish were anesthetized with ethyl P-aminobenzoate (benzocaine) and marked according to treatment group (25 fish each) with colored, 2.2-cm Petersen disk tags anchored below and slightly posterior to the dorsal fin. The fish were then injected with LH-RHa at 50.0, 5.0, or 0.5 μg/kg body weight; a combined injection of 0.5 mg/kg body weight of chum salmon (Oncorhynchus keta) lyophilized pituitary extract (SPE) and 5.0 μg/kg body weight of LH-RHa; or saline. Three days later, some groups were injected with 5.0 or 0.5 μg/kg body weight of LH-RHa (Table I).

The SPE and the LH-RHa were obtained from Syndel Laboratories Ltd., Vancouver, British Columbia, Canada. Hormone preparations were dissolved in sterile 0.6% saline and injected in volumes of 0.5 ml. All injections were administered intraperitoneally on the ventral side of the fish about midway between the vent and pelvic fins. Experimental fish were held in a concrete raceway (20.4 x
6.1 m) for the duration of the study. Water depth was maintained at 0.9 m. Water temperatures ranged from $5^\circ$ to $13.1^\circ$ C (mean, $9.2^\circ$ C). Fish were sorted and checked for ripeness every 3 to 4 days. After ovulation, 100 eggs were removed from each female and fertilized in separate containers. Individual males were used to fertilize eggs from an average of 5 females of several different treatment groups. Each group of eggs was then incubated in Heath Techna tray-type incubators at $10^\circ$ C. After 10 days, percent fertilization was determined by clearing the eggs in a solution of 10% acetic acid and then counting the number of eggs with developing embryos.

A total of four fish died prior to ovulation. Two died on day 0—probably due to handling stress—and were not considered in the analysis. The other two mortalities were taken into account when determining the cumulative percent ovulation of the population. A total of five fish from four different groups were unripe when spawned and were not considered in the analysis, as it was impossible to determine the date of ovulation. In all other fish, the eggs flowed freely from the body cavity when the females were spawned.

One-way analysis of variance (Snedecor and Cochran 1980) was conducted on the data for days to ovulation and fertility. The data were then subjected to Duncan's
Multiple Range Test to determine significant differences between specific treatments and between specific days.
Figure 8. Single injection experiment. Induced ovulation of coho salmon (*Oncorhynchus kisutch*). The numbers of fish which ovulated following injections of saline, lyophilized pituitary extract (SPE) and luteinizing hormone-releasing hormone (LH-RHa), or LH-RHa are expressed as cumulative percentages of their respective groups. Arrow indicates day on which the injections were administered.
Figure 8.
Figure 9. Serial injection experiment. Induced ovulation of coho salmon (*Oncorhynchus kisutch*). The numbers of fish which ovulated following injections of saline or luteinizing hormone-releasing hormone analogue (LH-RHa) are expressed as cumulative percentages of their respective groups. Arrows indicate the days on which the hormone injections were administered (saline was administered on day 0).
Figure 9.
RESULTS

Fish in groups treated with LH-RHa alone ovulated in significantly (P<0.01) fewer days than did fish in the control group (Table I). The mean days to ovulation for fish treated with SPE and LH-RHa were not significantly different from that for fish of the control group. Within 10 days after injection, 48% to 57% of the fish treated with LH-RHa alone—by either single or double injections and at all doses tested—had ovulated, whereas only 10% of the control group had ovulated (Figs. 8 and 9). Within 13 days after injection, 77% and 67% of the groups receiving single injections of LH-RHa at 50.0 and 5.0 μg/kg body weight, respectively, had ovulated; 91%, 84%, and 84% of the groups treated with two injections spaced three days apart of LH-RHa at 50.0 and 5.0, 5.0 and 5.0, and 0.5 and 0.5 μg/kg body weight, respectively, had ovulated; whereas only 47% of the control group had ovulated.

The mean percent fertilization of the eggs from the seven treatment groups ranged from 73% to 91% (Table I). The percent fertilization of eggs from individual females ranged from 6% to 100%. Although fertilization in the control group was slightly higher than in any treated group, this difference was not significant as judged by analysis of variance. Lower egg viabilities did not correlate directly
to specific males used for fertilization and, therefore, viabilities were considered dependent upon the condition of the females, although other factors may have influenced the fertilization of the eggs.

Within a treatment group, fertilization generally varied inversely with the number of days elapsed between treatment and ovulation, the means ranging from 65% to 98%. Eggs taken on day 7 had significantly (P<0.05) lower percent fertilization than eggs taken on any other day except day 35. Fertilization percentages were similar in all fish spawned on the same day, independent of treatment, except that on day 13 the fertility of eggs from the group receiving 50.0 μg/kg body weight of the LH-RHa was significantly (P<0.05) lower than that of eggs from the control group.
DISCUSSION

Intraperitoneal injections of LH-RHa significantly accelerated the date of ovulation in female coho salmon. The treatments were initiated 5 to 6 weeks prior to the projected peak spawning time of the hatchery population. Within two weeks, between 67% and 91% of the fish treated with LH-RHa alone had ovulated, suggesting that the analogue accelerated maturation up to one month prior to the normal peak spawning time and from 2 to 3 weeks prior to that of the control group. The LH-RHa, a synthetic hormone, successfully stimulated early ovulation without an initial "priming" injection of salmon gonadotropin or pituitary extract—an important finding due to the expense and difficulty in using the latter two natural products. That all of the groups treated with hormone analogue showed similar effects in relation to mean days to ovulation implies that LH-RHa is effective over a broad range of doses and that a lower dose than any we tested may be effective. The treatment of two serial injections of LH-RHa at 0.5 μg/kg body weight is the regime showing the greatest potential for use at a production level due to its cost-effectiveness, low mean days to ovulation, and the high fertility rate of eggs. It is apparent that this dosage sufficiently stimulates pituitary production and
secretion of gonadotropin (van der Kraak et al. 1983), which in turn mediates the final maturational process. The reason for the ineffectiveness of the combined SPE and LH-RHa treatment is at present undetermined.

The average percent fertilization was lower in eggs from fish treated with LH-RHa than in eggs of the control group, and was lowest in groups receiving single injections of the analogue. This relationship indicates that the serial injection technique, though offering little advantage in the magnitude of accelerated maturation, is superior to the single injection treatment in terms of egg fertility. Jalabert et al. (1978a) demonstrated that the administration of DHP to rainbow trout (Salmo gairdneri) resulted in precocious oocyte maturation in most fish, but only a few of the fish ovulated. These authors suggested that high levels of GtH were needed to complete maturation and induce ovulation. Perhaps the duration of elevated titers of gonadotropin is directly related to the fertility of the eggs.

Analysis of variance indicated that fertilization did not differ significantly between the treatment groups and control group; however, the average percent fertilization of the eggs from each group treated with LH-RHa was lower than that of the control group. Perhaps treatment with LH-RHa increases the incidence of extremely low egg viability in
certain individuals rather than causing a general decrease in the egg viability of each fish in the population. Only 3 fish (16%) in the control group had eggs with less than 80% fertilization, whereas the groups treated with LH-RHa had 5 (23%) to 9 (45%) fish with less than 80% fertilization of the eggs. The induction of precocious maturation may reach a point of diminishing returns due to lower percentages of fertilizable eggs. Fertility was lowest in eggs from the fish taken earliest in the experiment (day 7)--all of which received LH-RHa injections. It may be prudent to allow early fish an extra 24-72 hours after ovulation before taking the eggs, as the extra time might allow further maturation of the eggs and will also guard against human error in determining the ripeness of the fish.
IV. BIBLIOGRAPHY


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APPENDIX
V. Viabilities of eggs stripped from coho salmon at various times after ovulation

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V. Viabilities of eggs stripped from coho salmon at various times after ovulation.

INTRODUCTION

The use of hormones to accelerate final sexual maturation in salmonids has met with great success (Donaldson, et al. 1981; Hunter, et al. 1981; Sower, et al. 1982; Crim, et al. 1983, Sower, et al. 1984). However, little is known about the effects of these treatments on gamete quality. Sower et al. (1984) found that treatment of coho salmon, Oncorhynchus kisutch, with single injections of partially purified chinook salmon gonadotropin followed by single injections of the gonadotropin-releasing hormone analogue (LH-RHa) des-Gly^{10}[D-Ala^6]LH-RH-ethylamide accelerated maturation and that development of the eggs from these fish to eyed stage or later to ponding appeared to be equal to that of the control group. The highest mean percent viability in that study was 67.2 in eggs from control fish. As reported previously (see Chapter 3), treatment of coho salmon females with two injections of various dosages of LH-RHa accelerated final maturation, but the means for viability of the eggs were consistently lower for eggs from treated fish than for eggs from the control group—although the differences were not statistically significant.
In this study, females were allowed to retain their eggs after ovulation in order to determine if varying the amount of time between ovulation and egg-take had any effect on egg viability. Both LH-RHa-treated and untreated females were used to investigate the effect of holding the fish past ovulation had on egg viability.
MATERIALS AND METHODS

Adult female coho salmon from the seawater facility of Oregon Aqua Foods, Inc. at South Beach, Oregon were transported to the freshwater hatchery at Springfield, Oregon. On 17 October 1983, females exhibiting coloration indicative of advanced sexual maturity were separated from the rest of the broodstock population. These fish were anesthetized with ethyl P-aminobenzoate (benzocaine) and injected with LH-RHa at a dose of 0.5 µg/kg body weight. Three days later, the fish were injected with the same dosage of LH-RHa. Seven days after the initial injection, the fish were checked for ripeness. Approximately 100 females which had been designated as ripe (i.e. ovulated) were separated from the rest of the population. Of this group, 10 were removed and spawned. Approximately 100 eggs from each female were fertilized in separate containers with sperm pooled from four males. Each group of eggs was incubated at 10°C. After 10 days, percent fertilization was determined by clearing the eggs in a solution of 10% acetic acid and then counting the number of eggs with developing embryos.

Two days after the initial group of eggs were taken (and every two days thereafter), another group of ten females was sampled from the isolated population in the manner described
above. The last group of females was sampled 16 days after ovulation. On 12 December 1983, approximately 50 untreated females which had ovulated were separated from the rest of the broodstock population. Ten fish were removed and spawned, and the eggs were treated in the same manner as described above. Thereafter, groups of ten females were sampled in this way 4, 7, 15, and 20 days after ovulation and percent fertilization of each batch of eggs was determined.

One way analysis of variance (Snedecor and Cochran 1980) was conducted on the fertilization data with respect to days after ovulation within treated and untreated groups.
Figure 10. Mean (with standard error) and median percentages of viable eggs (i.e. with developing embryos 10 days after fertilization) from females stripped at various times after ovulation. The females were either a) treated with LH-RHa at 0.5 μg/kg body wt and ovulation took place on 24 October 1983; or b) untreated and ovulation took place on 12 December 1983.
Figure 10.
RESULTS AND DISCUSSION

The mean and median viabilities of eggs taken from LH-RHa-treated fish are presented in Fig. 10a. No significant differences were detected in the viabilities between eggs taken on the various days after ovulation. The values for the median viabilities of eggs were consistently higher than the values for the mean viabilities which indicates that the distribution of viabilities is skewed. In other words, the mean values have been disproportionately influenced by a few very low viabilities. The skewness of the distribution is readily apparent in an examination of the viabilities of eggs from all fish treated with LH-RHa. The mean percent viability of the eggs was 81.5 ± 2.3, whereas the median percent viability was 90.2. Almost 71% of the viabilities were greater than or equal to the mean value.

Three incidences of overripe (i.e. water hardened) eggs occurred in the group treated with LH-RHa—in one fish on day 12 and in two fish on day 14 after ovulation. The viabilities of these eggs were less than or equal to 45.9%. No overripe eggs were observed in the 10 fish spawned 16 days after ovulation. Eleven of the 106 females in the treated group died during the course of the experiment, representing 10.4% of the population. Mortalities began
appearing 3 days after ovulation.

The mean and median viabilities of eggs taken from untreated fish are presented in Fig. 10b. No significant differences in the viabilities between days were detected and the median values were generally closer to the mean values for untreated fish than for LH-RHa-treated fish. The mean viabilities of eggs remained high (over 90%) for the duration of the experiment, 20 days after ovulation.

Nomura et al. (1974) described 4 distinct stages of overripeness in eggs from rainbow trout, Salmo gairdneri. Sakai et al. (1975) showed that the percentage of eyed eggs and percentage of hatched rainbow trout exceeded 70% in those eggs which had been stripped by the 10th day after ovulation; however, the viabilities at the same stages declined rapidly in eggs stripped between 10 and 20 days after ovulation and reached 0% for eggs stripped one month after ovulation. Takashima et al. (1975) described histologically some of the profound changes taking place in overripe female rainbow trout and Craik and Harvey (1984), examining some of the biochemical changes occurring with overripening in eggs of rainbow trout, found that free, bound, and total lipid varied significantly between ripe and overripe eggs (i.e. eggs taken 30 or more days after ovulation) as did protein phosphorus, lipid phosphorus, iron and calcium.
The results of the present study on coho salmon agree in general with that which has been reported in the literature. Eggs of high viability (i.e. showing embryonic development) were obtained from females that were treated with LH-RHa to induce precocious final maturation up to 16 days after ovulation and from untreated females up to 20 days after ovulation. However, in hormone-treated fish, the mortality of adult females was high, commencing three days after ovulation, and incidences of water hardened eggs appeared 12 days after ovulation. Thus, although it appears that females treated with LH-RHa can be held for long periods of time after ovulation in order to satisfy the need for a flexible strategy of taking eggs, some adverse effects can occur, such as mortality and water hardened eggs. Untreated females do not appear to suffer in the same magnitude from these maladies, although it is difficult to determine from this study if the higher egg viabilities were due to not receiving hormone treatments or due to some other effect, such as later-spawning females having eggs with different characteristics than early-spawning females.
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